Increased risk and mortality of invasive pneumococcal disease in HIV-exposed-uninfected infants <1 year of age in South Africa, 2009-2013

Claire von Mollendorf MBBCh, MSc [1,2], Anne von Gottberg MBBCh, PhD [1,3], Stefano Tempia DVM, PhD [1,4,5], Susan Meiring MBChB [6], Linda de Gouveia ND MT (Micro) [1], Vanessa Quan MBBCh, MPH [6], Sarona Lengana MBBCh [1], Theunis Avenant MBBCh, FCPaed (SA) [7], Nicolette du Plessis MBBCh, FCPaed (SA) [7], Brian Eley MBBCh, FCPaed (SA) [8], Heather Finlayson MBChB, FCPaed (SA) [9], Gary Reubenson MBBCh, FCPaed (SA) [10], Mamokgethi Moshe MBBCh, FCPaed (SA) [11], Katherine L. O'Brien MD, MPH [12], Keith P. Klugman MBBCh, PhD [13], Cynthia G. Whitney MD, MPH [14] and Cheryl Cohen MBBCh, PhD [1,2], for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA)

[1] Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, a division of the National Health Laboratory Service, Johannesburg, South Africa;

[2] School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa;

[3] Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, School of Pathology, University of the Witwatersrand, Johannesburg, South Africa;

[4] Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America (USA);

[5] Influenza Division, Centers for Disease Control and Prevention, Pretoria, South Africa;

[6] Division of Public Health Surveillance and Response, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa;

[7] Steve Biko (Pretoria Academic Hospital) and Kalafong Hospital, Pediatric Infectious Diseases Unit, University of Pretoria, Pretoria, Gauteng, South Africa;

[8] Red Cross War Memorial Children's Hospital, and the Department of Paediatrics and Child Health, University of Cape Town, Cape Town, Western Cape, South Africa;

[9] Tygerberg Hospital and Department of Paediatrics and Child Health, Stellenbosch University, Cape Town, Western Cape, South Africa;

[10] Rahima Moosa Mother and Child Hospital, Department of Paediatrics and Child Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, Gauteng, South Africa;

[11] Dr George Mukhari Hospital, Department of Paediatrics and Child Health, Medunsa University, Gauteng Province, South Africa;

[12] Johns Hopkins Bloomberg School of Public Health, International Vaccine Access Center, Department of International Health, Baltimore, Maryland, USA;

[13] Hubert Department of Global Health, Rollins School of Public Health, and Division of Infectious Diseases, School of Medicine, Emory University, Atlanta, USA;

[14] National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

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Corresponding author: Claire von Mollendorf, Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, 1 Modderfontein Road, NHLS, Sandringham, 2193, Gauteng, South Africa; Telephone: 27 11 3866321, Fax: 27 11 3866580, Cell: +27 (0)82 828 8134, E-mail: clairevm@nicd.ac.za

Alternative corresponding author: Cheryl Cohen, Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, 1 Modderfontein Road, NHLS, Sandringham, 2193, Gauteng, South Africa; Telephone: 27 11 3866593, Fax: 27 11 8829979, Cell: +27 (0)82 803 8093, E-mail: cherylc@nicd.ac.za

Key points (27 words):

HIV-exposed-uninfected children have an increased risk of invasive pneumococcal disease related hospitalizations, with increased in-hospital mortality in infants <6 months of age when compared with unexposed children.

Abstract

Background

High antenatal HIV seroprevalence rates (~30%) with low perinatal HIV transmission rates (2.5%) due to HIV prevention of mother-to-child transmission program improvements in South Africa, has resulted in increasing numbers of HIV-exposed-uninfected (HEU) children. We aimed to describe the epidemiology of invasive pneumococcal disease (IPD) in HEU infants.

Methods

We conducted a cross-sectional study of infants aged <1 year with IPD enrolled in a national, laboratory-based surveillance program for incidence estimations. Incidence was reported for two time points, 2009 and 2013. At enhanced sites we collected additional data including HIV status and in-hospital outcome.

Results

We identified 2099 IPD cases in infants from 2009-2013 from all sites. In infants from enhanced sites (n=1015), 92% had known HIV exposure status and 86% had known outcomes. IPD incidence was highest in HIV-infected infants, ranging from 272-654/100,000 population between time points (2013 and 2009), followed by HEU (33-88/100,000) and

HIV-unexposed-uninfected (HUU) infants (18-28/100,000). Case fatality rate in HEU (29%, 74/253) was intermediate between HUU (25%, 94/377) and HIV-infected infants (34%, 81/242). When restricted to cases <6 months of age, HEU infants (37%, 59/175) were at significantly higher risk of dying than HUU infants (32%, 51/228; Adjusted relative risk ratio = 1.76 [95% confidence interval 1.09-2.85]).

Discussion

HEU infants are at increased risk of IPD and mortality from IPD compared with HUU children, especially as young infants. HEU infants, whose numbers will likely continue to increase, should be prioritized for interventions such as pneumococcal vaccination along with HIV-infected infants and children.

Introduction

Prevention of mother-to-child transmission (PMTCT) HIV programs have expanded over the last decade in many countries. South Africa reported a decrease in mother-to-child HIV transmission rates from 12% in 2007 to 2.7% in 2011 [1] and 2.5% during 2012/2013 [2], despite a relatively constant prevalence of HIV in pregnant women of around 30%. This has resulted in an increasing number of HIV-exposed-uninfected (HEU) infants, especially in countries with elevated HIV prevalence like South Africa.

All-cause hospitalization rates and complicated hospital admissions are more frequent in HEU than HIV-unexposed-uninfected (HUU) infants [3, 4]. Some infectious diseases, including respiratory tract infections [5], are more common and often more severe amongst HEU than HUU children. When compared with HIV-infected children, one study reported

similar rates of pneumonia and bacterial meningitis in HEU children, but higher rates of gastroenteritis and sepsis [6].

Some studies report higher mortality rates in HEU than HUU infants [7, 8] while others show no difference [9, 10]. In contrast, studies consistently report higher mortality rates in HIV-infected versus HEU or HUU infants [7]. Risk factors for mortality in HEU children include advanced maternal HIV disease [11, 12], malnutrition [8], severe pneumonia and bacterial meningitis [6]. Mortality among HEU children peaks in younger infants (3–6 months) with death being predominantly associated with lower respiratory tract infections [8]. Within the general population, the pneumococcus is estimated to cause 30-40% of childhood community-acquired pneumonia cases [13]

There are no published data evaluating or quantifying the risk of hospitalization or mortality associated with invasive pneumococcal disease (IPD) among HEU children. We aimed to describe the epidemiology of IPD from 2009 to 2013 in South African HEU infants <1 year of age, compared to the epidemiology of IPD in similarly aged HUU and HIV-infected infants.

Methods (see supplementary appendix for additional methods)

Study design and setting

Children hospitalized from 2009 through 2013 with laboratory-confirmed IPD were prospectively identified by a national, laboratory-based, active surveillance program for *Streptococcus pneumoniae*. Over 200 routine hospital-based diagnostic laboratories (enhanced and non-enhanced hospital sites) systematically report IPD cases of all ages to the surveillance program. For the subset of cases occurring at 25 enhanced sentinel hospital

sites, located in all nine provinces, dedicated study surveillance officers collect additional clinical and demographic information.

Study population

We included all infants <1 year of age with IPD from 2009 through 2013. For incidence calculations, infants from enhanced and non-enhanced sites were included. For analyses of factors associated with HIV exposure/infection status and mortality, only infants from enhanced sites with known HIV exposure status and in-hospital outcome were included.

Case definitions

IPD cases were defined as *S. pneumoniae* identified from normally sterile site (e.g. cerebrospinal fluid (CSF), blood, joint fluid, pleural fluid) specimens at participating sites. HUU infants were defined as infants with documented negative maternal HIV status at birth or time of illness, with or without a negative HIV enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) result for the infant. HEU infants were defined as infants who had a negative HIV PCR result with known positive maternal HIV status (verbal or documented positive result) or infants with a positive HIV ELISA result and negative HIV PCR result. HEU infants, who had symptoms suggestive of HIV at the current admission, were retested. HIV-infected infants were defined as infants with a positive HIV PCR result before or at time of illness.

Incidence

We calculated annual incidence of pneumococcal disease from 2009 through 2013 for infants <1 year of age, by HIV infection/exposure status, by dividing number of laboratory-

confirmed IPD cases reported each year in each category (HEU, HUU and HIV) by mid-year population estimates for each group. Population denominators were obtained from the THEMBISA model [14]. Due to significant decreasing trends in IPD incidence rates from 2009 through 2013, resulting from progressive pneumococcal conjugate vaccine (PCV) introduction and HIV-related interventions [15], we only presented data from two time points, pre- (2009) and post-vaccine (2013) introduction.

As HIV infection/exposure status information was only available for cases identified at enhanced sites, we assumed a similar prevalence of HIV infection and exposure amongst cases with unknown (from non-enhanced sites) status as that found at enhanced sites. We calculated relative risk of IPD hospitalization comparing HEU to HUU and HIV-infected children. Confidence intervals were calculated using Poisson distribution for incidence rates (IR) and incidence rate ratios (IRR).

Factors associated with HIV exposure status and death

We included infants <1 years of age with IPD from enhanced sites only, from 2009 through 2013, and developed two multivariable models to identify factors associated with outcome variables: (i) HIV infection/exposure status; and (ii) mortality. Multinomial regression was used for comparison of factors associated with HIV infection/exposure. Multinomial regression allows modeling of outcome variables with more than two categories and relates the probability of being in category *j* to the probability of being in a baseline category. A complete set of coefficients are estimated for each of the *j* levels being compared with the baseline and the effect of each predictor in the model is measured as relative risk ratio (RRR). HEU cases were used as the referent group and compared with HUU and HIV-infected

cases so that all described differences would be related to exposed children. The model to assess factors associated with mortality used logistic regression and was presented stratified by age (<6 and 6-<12 months) as there was significant interaction between age and HIV infection/exposure status. Statistical analysis was implemented using Stata version 12 (StataCorp Inc., College Station, Texas, USA).

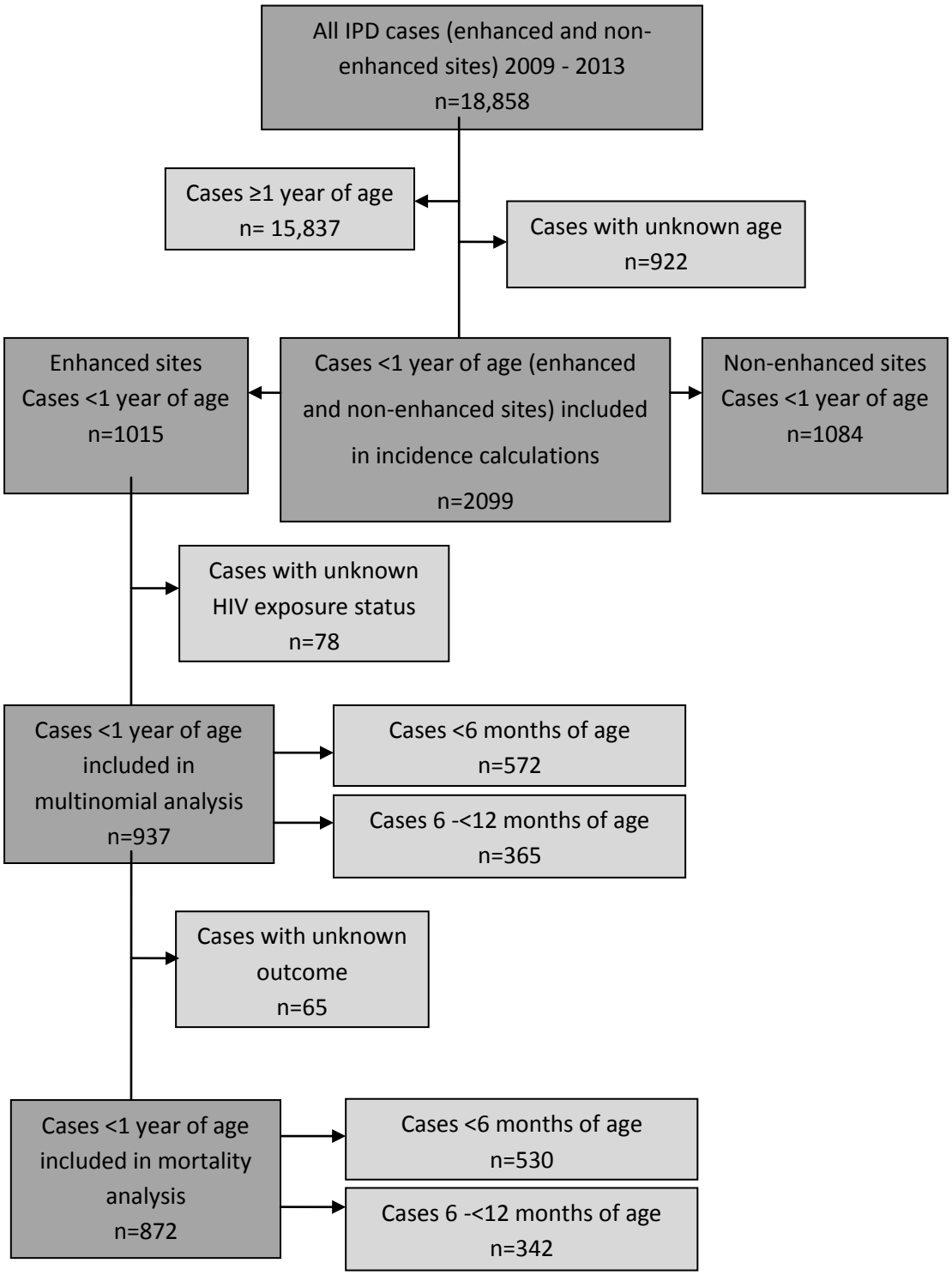
Ethics

Ethics approval was obtained for GERMS-SA surveillance (M081117) from the Human Research Ethics Committee (Medical), University of the Witwatersrand, Johannesburg, South Africa and other local hospital or provincial ethics committees, as required.

Results

We identified 2099 IPD cases in infants <1 year of age from 2009 through 2013 from all sites (Figure 1). Enhanced sites, predominantly regional and tertiary hospitals, contributed about 50% (n=1015) of all isolates received. Non-enhanced sites included district, regional and tertiary public hospitals, private hospitals and clinics. Regional and tertiary hospitals contributed 73% (787/1084) of isolates sent from non-enhanced sites. In cases from enhanced sites, 92% (937/1015) had known HIV exposure/infection status and 86% (872/1015) had known in-hospital outcomes. Compared with non-enhanced sites, enhanced site cases were more likely to be diagnosed on positive blood (OR 1.24 95% CI 1.04-1.48) or other specimen culture (OR 2.36 95% CI 1.39-4.03), as compared to CSF, as these specimens were more likely to be done at enhanced sites. Age distribution (<6 and 6-<12 months) did not differ (OR 1.10 95% CI 0.92-1.31) between enhanced and non-enhanced sites (data not shown).

Figure 1: Patients with invasive *Streptococcus pneumoniae* disease (IPD) reported from GERMS-SA surveillance sites, South Africa, 2009-2013



Breastfeeding information was only available for children enrolled in a nested case-control study. In the first 4 months of life, 33% (30/90) of HEU children, 81% (119/147) of HUU children and 56% (43/77) of HIV-infected children were breastfed. Seventy-six percent (n=207) of all HEU children from enhanced sites had known HIV testing dates; only 61 (29%) were tested more than a month prior to admission and 34 (of the 61) had a known feeding status, with only 7 being breastfed.

Serotype distribution differed by known HIV status for enhanced site patients. Across all years, isolates from HIV-infected cases were more likely to be vaccine serotypes (71%, 160/225), than isolates from HEU cases (57%, 139/244; p<0.001); while prevalence was similar between isolates from HEU and HUU cases (56%, 198/356; p=0.88). A similar proportion of cases were isolated from CSF and blood cultures in HIV-infected (33%, 84/257 and 65%, 167/257) and HEU cases (38%, 103/273 and 61%, 166/273), while proportions differed in HUU cases (CSF 43%, 175/407; blood culture 51%, 207/407; p<0.001). The proportion of vaccine-type IPD decreased in all 3 groups between 2009 and 2013: for HEU infants vaccine-type disease was 79% (48/61) and 30% (12/40) (p<0.001); for HUU infants 72% (72/100) and 23% (15/64) (p<0.001), and for HIV-infected infants 85% (75/88) and 35% (7/20) (p<0.001), respectively.

Incidence rates

In 2009 (Table 1) IPD incidence in the <1 year age group was higher in HIV-infected compared with HUU (20-fold) and HEU infants (7-fold). HEU infants also had a 3-fold higher incidence of IPD than HUU infants. When stratified into two age groups, incidence was

Table 1: IPD incidence rates and incident rate ratios between groups of HIV-infected (HI), HIV-exposed-uninfected (HEU), and HIV-unexposed-uninfected (HUU) infants <12 months, <6 months and 6-<12 months of age, in South Africa, 2009 (pre-vaccine) and 2013 (post-vaccine)

	Incidence rates/100 000 population			Incidence rate ratio (IRR)		
	HI (95% CI)	**HEU (95% CI)**	**HUU (95% CI)**	**HI/HEU (95% CI)**	**HI/HUU (95% CI)**	**HEU/HUU (95% CI)**
2009						
<6 months	1156 (972-1364)	112 (94-132)	31 (26-37)	10.3 (8.1-13.1)	37.0 (29.0-47.2)	3.6 (2.8-4.6)
6-<12 months	467 (394-551)	59 (46-75)	26 (21-31)	7.9 (5.9-10.8)	18.0 (14.0-23.3)	2.3 (1.7-3.1)
<12 months	654 (579-736)	88 (76-100)	28 (25-32)	7.5 (6.2-9.0)	23.1 (19.4-27.6)	3.1 (2.6-3.7)
2013						
<6 months	581 (389-835)	57 (46-71)	21 (17-26)	10.1 (6.4-15.7)	27.2 (17.2-41.7)	2.7 (2.0-3.7)
6-<12 months	149 (92-227)	11 (6-18)	14 (11-18)	13.9 (6.7-29.5)	10.4 (6.0-17.4)	0.8 (0.4-1.4)
<12 months	272 (203-357)	33 (26-40)	18 (15-21)	8.4 (5.9-12.0)	15.0 (10.7-20.6)	1.8 (1.4-2.3)

similarly highest in HIV-infected, intermediate in HEU and lowest in HUU infants (Table 1). By 2013, although incidence rates had decreased, due to PCV and HIV interventions, in all groups compared with 2009, relative trends in incidence by HIV-exposure/infection status were similar. In 2013, in the 6-<12 month age group, incidence was similar in HEU and HUU cases, but case numbers were small in this age group limiting the ability to detect relative differences in rates. Incidence and IRR were higher in the <6 month age group than in the 6-<12 month age group regardless of HIV status (Table 1).

Factors associated with HIV-exposure and infection status

For cases <1 year of age, with known outcomes and HIV status, the overall case-fatality ratio was high (29%, 249/872), with mortality in HEU (29%, 74/253) intermediate between HUU (25%, 94/377) and HIV-infected infants (34%, 81/242) (p=0.07) (supplementary Table 1 (S1)). When comparing HEU (n=273) with HUU (n=407) cases on multivariable analysis, HUU cases were twice as likely to be >6 months of age or to have meningitis when compared to pneumonia, but less likely to be of black race.

On multivariable analysis, HIV-infected infants (n=257) (Table S1) were more likely to be >6 months of age, be infected with penicillin non-susceptible *S. pneumoniae*, have used cotrimoxazole prophylaxis in the last month and have died when compared with HEU children. In addition, HIV-infected infants were less likely to have underlying conditions other than HIV and malnutrition.

When we restricted the analysis to cases <6 months of age (Table 2), on multivariable analysis, HUU cases were at significantly lower risk of dying during the IPD episode, had a

Table 2: Univariate and multivariate multinomial logistic regression model showing comparison of demographic, socio-economic characteristics, and underlying conditions in HIV-exposed-uninfected (HEU), HIV-unexposed-uninfected (HUU) and HIV-infected invasive pneumococcal disease cases at enhanced GERMS-SA sites in South Africa, <6 months, 2009-2013 (n=572)

	HEU cases Reference	HUU cases			HIV-infected cases		
	n/N (%)	n/N (%)	RRR[a] (95%CI)	ARRR[b] (95%CI)	n/N (%)	RRR[a] (95%CI)	ARRR[b] (95%CI)
Demographics and socioeconomic characteristics							
Black Race	172/176 (97.7)	203/239 (84.9)	0.13 (0.05-0.38)	0.15 (0.05-0.45)	129/130 (99.2)	3.00 (0.33-27.16)	3.48 (0.37-32.69)
Length of hospital stay:							
<4 days	48/174 (27.6)	47/227 (20.7)	Reference		37/127 (29.1)	Reference	
4-14 days	90/174 (51.7)	102/227 (44.9)	1.16 (0.71-1.89)		60/127 (47.2)	0.86 (0.50-1.48)	
≥15 days	36/174 (20.7)	78/227 (34.4)	2.21 (1.26-3.89)		30/127 (23.6)	1.08 (0.57-2.06)	
Medical conditions and treatment							
Underlying conditions[c]	21/151 (13.9)	22/209 (10.5)	0.73 (0.38-1.38)		4/113 (3.5)	0.23 (0.08-0.68)	
Malnutrition[d]	58/168 (34.5)	81/221 (36.7)	1.10 (0.72-1.67)	1.20 (0.72-2.02)	80/122 (65.6)	3.61 (2.21-5.90)	3.19 (1.80-5.64)
Previous hospital admission in last 12 months	32/172 (18.6)	42/224 (18.8)	1.01 (0.61-1.68)		41/119 (34.5)	2.30 (1.34-3.94)	
In-hospital mortality	59/175 (33.7)	51/228 (22.4)	0.57 (0.36-0.88)	0.46 (0.26-0.81)	50/126 (39.7)	1.29 (0.80-2.08)	1.55 (0.87-2.76)
Previous IPD infection[e]	2/189 (1.1)	4/249 (1.6)	1.53 (0.28-8.42)		7/134 (5.2)	5.15 (1.05-25.21)	
Cotrimoxazole prophylaxis	26/170 (15.3)	0/249 (0.0)	Not calculated		37/110 (33.6)	2.81 (1.58-4.99)	
Treated for tuberculosis	5/174 (2.9)	6/229 (2.6)	0.91 (0.27-3.03)		12/116 (10.3)	3.90 (1.34-11.39)	
Pneumococcal isolate characteristics							

Penicillin non-susceptible[f]	69/157 (43.9)	69/201 (34.3)	0.67 (0.43-1.02)	0.61 (0.38-0.99)	64/105 (61.0)	1.99 (1.20-3.29)	1.79 (1.03-3.09)
Vaccine serotypes[g]	94/171 (55.0)	110/217 (50.7)	0.84 (0.56-1.26)		77/111 (69.4)	1.86 (1.12-3.07)	
Clinical syndrome and specimen type							
Clinical syndrome							
- Pneumonia	74/183 (40.4)	77/239 (32.2)	Reference	Reference	69/131 (52.7)	Reference	Reference
- Meningitis	92/183 (50.3)	129/239 (54.0)	1.35 (0.88-2.04)	1.89 (1.12-3.20)	47/131 (35.8)	0.55 (0.34-0.89)	0.86 (0.46-1.57)
- Bacteremia	17/183 (9.3)	33/239 (13.8)	1.87 (0.96-3.63)	1.96 (0.86-4.44)	15/131 (11.5)	0.95 (0.44-2.04)	1.81 (0.75-4.36)
Specimen type[h]							
- Blood culture	105/189 (55.6)	117/249 (47.0)	Reference		89/134 (66.4)	Reference	
- Cerebrospinal fluid	81/189 (42.9)	123/249 (49.4)	1.36 (0.93-2.00)		42/134 (31.3)	0.61 (0.38-0.98)	
- Other	3/189 (1.6)	9/249 (3.6)	2.69 (0.71-10.21)		3/134 (2.2)	1.18 (0.23-5.99)	

Only variables significant on univariate and multivariable analysis are shown. Variables not included in table: sex, Pitt bacteraemia score, antibiotics in last 24 hours, antibiotics in last 2 months and vaccination status.

[a]Relative risk ratio; [b]Adjusted relative risk ratio; [c]Asplenia, including asplenia or sickle cell anemia; chronic illness, including chronic lung, renal, liver, cardiac disease and diabetes; other immunocompromising conditions (excluding HIV), including organ transplant, primary immunodeficiency, immunotherapy and malignancy; and other risk factors, including head injury with possible CSF leak, neurological disorders, burns and chromosomal abnormalities. Excludes malnutrition; [d]Malnutrition was classified as children with weight-for-age Z-score < -2 (WHO child growth standards 2009) and/or children with nutritional edema; [e] Previously diagnosed with IPD (invasive pneumococcal disease) more than 21 days prior to this episode; [f] Penicillin non-susceptible MIC ≥ 0.12 µg/mL; [g]Vaccine serotypes were considered as serotypes in the 13-valent pneumococcal conjugate vaccine; [h]Elected to use clinical diagnosis rather than specimen type in multivariable model

Table 3: Univariate and multivariate multinomial logistic regression model showing comparison of demographic, socio-economic characteristics, and underlying conditions in HIV-exposed-uninfected (HEU), HIV-unexposed-uninfected (HUU) and HIV-infected invasive pneumococcal disease cases at enhanced GERMS-SA sites in South Africa, 6-<12 months, 2009-2013 (n=365)

	HEU cases Reference	HUU cases			HIV-infected cases		
	n/N (%)	n/N (%)	RRR[a] (95%CI)	ARRR[b] (95%CI)	n/N (%)	RRR[a] (95%CI)	ARRR[b] (95%CI)
Demographics and socioeconomic characteristics							
Black Race	78/81 (96.3)	132/154 (85.7)	0.23 (0.07-0.80)	0.15 (0.03-0.70)	114/117 (97.4)	1.46 (0.29-7.43)	0.82 (0.09-7.23)
Medical conditions and treatment							
Underlying conditions[c]	15/77 (19.5)	41/141 (29.1)	1.69 (0.87-3.31)	1.99 (0.90-4.37)	11/103 (10.7)	0.49 (0.21-1.15)	0.29 (0.09-0.87)
Malnutrition[d]	31/78 (39.7)	54/147 (36.7)	0.88 (0.50-1.55)	0.68 (0.35-1.34)	73/109 (67.0)	3.07 (1.68-5.63)	2.36 (1.13-4.96)
Previous hospital admission in last 12 months	19/74 (25.7)	57/148 (38.5)	1.81 (0.98-3.36)		51/111 (45.9)	2.46 (1.29-4.67)	
In-hospital mortality	15/78 (19.2)	43/149 (28.9)	1.70 (0.88-3.31)	3.38 (1.34-8.53)	31/116 (26.7)	1.53 (0.76-3.08)	2.82 (1.02-7.78)
Pitt bacteremia score (≥4)[e]	9/76 (11.8)	21/151 (13.9)	1.20 (0.52-2.77)	0.62 (0.21-1.84)	5/113 (4.4)	0.34 (0.11-1.07)	0.13 (0.02-0.72)
Antibiotics in last 2 months[f]	7/73 (9.6)	37/145 (25.5)	3.23 (1.36-7.66)		23/104 (22.1)	2.68 (1.08-6.63)	
Cotrimoxazole prophylaxis	8/73 (11.0)	0/158 (0.00)	Not calculated	Not calculated	50/111 (45.1)	6.66 (2.92-15.17)	11.18 (4.04-30.91)
Treated for tuberculosis	4/75 (5.3)	4/148 (2.7)	0.49 (0.11-2.02)		18/112 (16.1)	3.40 (1.10-10.48)	

Pneumococcal isolate characteristics					
Penicillin non -susceptible[g]	30/62 (48.4)	68/130 (52.3)	1.17 (0.64-2.14)	74/111 (66.7)	2.13 (1.13-4.03)
Specimen type					
Specimen type					
- Blood culture	61/84 (72.6)	90/158 (57.0)	Reference	78/123 (63.4)	Reference
- Cerebrospinal fluid	22/84 (26.2)	52/158 (32.9)	1.60 (0.88-2.90)	42/123 (34.1)	1.49 (0.81-2.76)
- Other	1/84 (1.2)	16/158 (10.1)	10.84 (1.40-83.92)	3/123 (2.4)	2.35 (0.24-23.12)

Only variables significant on univariate and multivariable analysis are shown. Variables not included in table: sex, length of hospital stay, antibiotics in last 24 hours, previous IPD infection, vaccine serotypes, vaccination status and clinical syndrome; [a]Relative risk ratio; [b]Adjusted relative risk ratio; [c]Asplenia, including asplenia or sickle cell anemia; chronic illness, including chronic lung, renal, liver, cardiac disease and diabetes; other immunocompromising conditions (excluding HIV), including organ transplant, primary immunodeficiency, immunotherapy and malignancy; and other risk factors, including head injury with possible CSF leak, neurological disorders, burns and chromosomal abnormalities. Excludes malnutrition; [d]Malnutrition was classified as children with weight-for-age Z-score < -2 (WHO child growth standards 2009) and/or children with nutritional edema; [e]Pitt bacteremia score calculated using temperature, hypotension, mechanical ventilation, cardiac arrest and mental status. Severe disease defined as score of ≥4 points; [f]Any antibiotics used in 2 months prior to admission**;** [g]Penicillin non-susceptible MIC ≥ 0.12 µg/mL.

decreased risk of IPD caused by a penicillin non-susceptible strain, and an increased risk of meningitis compared with pneumonia than HEU children with IPD. HIV-infected infants with IPD were more likely to have disease caused by a penicillin non-susceptible strain and be malnourished than HEU infants with IPD.

For cases 6-<12 months of age (Table 3), on multivariable analysis HUU infants were less likely to be of black race and at significantly increased risk of dying from their IPD episode than HEU children. HIV-infected cases were more likely to be malnourished and die than HEU cases, despite having less severe disease at time of presentation, as assessed with Pitt bacteremia score, and fewer underlying conditions other than HIV. Amongst HUU cases, underlying conditions were significantly more common in cases aged 6-<12 months (41/141, 29.1%) as compared to younger cases (22/209, 10.5%; p<0.001).

Factors associated with case fatality

On multivariable analysis to explore factors associated with death, in cases <6 months (Table 4) being of black race, malnourished, HEU or HIV-infected and having meningitis (compared with pneumonia) was associated with an increased odds of death. In the 6-<12 month age group (Table 5) cases with malnutrition (compared to no malnutrition), those with meningitis (compared with pneumonia) and HUU cases (compared with HEU cases) had increased odds of death.

Discussion

In South Africa, HIV-infected paediatric numbers continue to drop due to PMTCT improvements [1]; however numbers of HEU children remain high and are growing. We

Table 4: Univariate and multivariable analysis showing factors associated with mortality in invasive pneumococcal disease cases <6 months of age in South Africa, 2009-2013 (n=530)

		Univariate analysis[a]			Multivariable analysis[a]	
		CFR n/N (%)	OR[b] (95% CI)	P-value	aOR[c] (95% CI)	P-value
Demographics and socioeconomic characteristics						
Race	Non-black	5/44 (11.4)	Reference	0.03	Reference	0.02
	Black	164/501 (32.7)	3.31 (1.14-9.64)		4.14 (1.22-14.04)	
Length of hospital stay	<4 days	121/140 (86.4)	Reference	<0.001		
	4-14 days	35/254 (13.8)	0.02 (0.01-0.04)			
	≥15 days	9/144 (6.3)	0.01 (0.004-0.02)			
Medical conditions and treatment						
Malnutrition[d]	No	77/292 (26.4)	Reference	0.27	Reference	0.03
	Yes	75/218 (34.4)	1.26 (0.84-1.90)		1.63 (1.05-2.53)	
Pitt bacteremia score[e]	0-3	109/440 (24.8)	Reference	<0.001		
	≥4	46/73 (63.0)	5.03 (2.92-8.65)			
Any antibiotics used in last 24 hours[f]	No	114/439 (26.0)	Reference	0.02		
	Yes	23/58 (39.7)	2.05 (1.15-3.66)			
HIV status[g]	HUU	51/228 (31.9)	Reference	0.002	Reference	0.007

	HEU	59/175 (36.9)	1.77 (1.13-2.75)		1.76 (1.09-2.85)	
	HIV-infected	50/126 (31.3)	2.28 (1.42-3.67)		2.25 (1.32-3.82)	
Clinical syndrome						
Clinical syndrome	Pneumonia	64/223 (28.7)	Reference	0.10	Reference	0.009
	Meningitis	90/260 (34.6)	1.47 (0.98-2.22)		1.92 (1.22-3.03)	
	Bacteraemia	17/66 (25.8)	0.89 (0.46-1.74)		0.92 (0.45-1.88)	

[a]Only variables significant on univariate and multivariable analysis are shown. Variables not included in table: age group, sex, wood fire in the home, referral, previous admission, low birth weight, underlying conditions, antibiotics in last 2 months, penicillin non-susceptibility, previous IPD infection, cotrimoxazole prophylaxis, TB treatment and vaccination status; [b]Odds ratio; [c]Adjusted odds ratio; [d]Malnutrition was classified as children with weight-for-age Z-score < -2 (WHO child growth standards 2009) and/or children with nutritional edema; [e]Pitt bacteremia score calculated using temperature, hypotension, mechanical ventilation, cardiac arrest and mental status. Severe disease defined as score of ≥4 points; [f]Any antibiotics used in 24 hours prior to admission; [g]HEU=HIV-exposed-uninfected, HUU=HIV-unexposed-uninfected

Table 5: Multivariable analysis showing factors associated with mortality in invasive pneumococcal disease cases 6-<12 months of age in South Africa, 2009-2013 (n=342)

		Univariate analysis[a]			Multivariable analysis[a]	
		CFR n/N (%)	**OR[b] (95% CI)**	**P-value**	**aOR[c] (95% CI)**	**P-value**
Demographics and socioeconomic characteristics						
Length of hospital stay	<4 days	67/91 (73.6)	Reference	<0.001		
	4-14 days	15/164 (9.2)	0.03 (0.02-0.07)			
	≥15 days	15/99 (15.2)	0.07 (0.03-0.15)			
Medical conditions						
Malnutrition[d]	No	32/177 (18.1)	Reference	0.003	Reference	0.001
	Yes	48/158 (30.4)	2.30 (1.32-4.01)		2.58 (1.45-4.60)	
Pitt bacteremia score[e]	0-3	66/309 (21.4)	Reference	<0.001		
	≥4	28/41 (68.3)	8.66 (3.94-19.05)			
HIV status[f]	HUU	43/149 (28.9)	Reference	0.29	Reference	0.06
	HEU	15/78 (19.2)	0.59 (0.30-1.14)		0.46 (0.22-0.98)	
	HIV-infected	31/116 (26.7)	0.90 (0.52-1.55)		0.55 (0.29-1.04)	
Clinical syndrome						
Clinical syndrome	Pneumonia	37/164 (22.6)	Reference	0.04	Reference	0.03

Meningitis	48/130 (36.9)	1.82 (1.06-3.10)	2.16 (1.19-3.92)
Bacteraemia	16/67 (23.9)	0.88 (0.42-1.84)	1.03 (0.48-2.24)

[a]Only variables significant on univariate and multivariable analysis are shown. Variables not included in table: age group, sex, race, wood fire in the home, referral, previous admission, low birth weight, underlying conditions, antibiotics in last 24 hours, antibiotics in last 2 months, penicillin non-susceptibility, previous IPD infection, cotrimoxazole prophylaxis, TB treatment and vaccination status; [b]Odds ratio; [c]Adjusted odds ratio; [d]Malnutrition was classified as children with weight-for-age Z-score < -2 (WHO child growth standards 2009) and/or children with nutritional edema; [e]Pitt bacteremia score calculated using temperature, hypotension, mechanical ventilation, cardiac arrest and mental status. Severe disease defined as score of ≥4 points; [f]HEU=HIV-exposed-uninfected, HUU=HIV-unexposed-uninfected

have shown that these HIV-exposed but uninfected children are twice as likely to have an IPD-associated hospitalization; and that HEU children aged <6 months are less likely to survive an IPD episode than HUU children. It is important to prioritize and continue targeting these HEU children for public health interventions such as PCV vaccination.

Following PCV introduction into the national immunization program in South Africa, a significant reduction in vaccine-type disease in HIV-infected and –uninfected children was observed [15]. In our study, we similarly, observed a reduction in the estimated incidence of IPD in HEU children from 2009-2013. While other interventions such as improvements in maternal immune status [12] may have contributed to this, it is likely that the bulk of this reduction resulted from the introduction of PCV [15]. A case-control study from South Africa showed that PCV, when given in the routine program, was highly effective in HEU children [16]. Despite generally lower prevaccination antibody levels, HEU children respond quantitatively as well as HUU infants to routine immunization program vaccinations like tetanus, pertussis, *Haemophilus influenzae* type b and hepatitis B [17]. In contrast, PCV functional assays have shown that HEU children require higher antibody concentrations for effectiveness against certain pneumococcal serotypes [18].

In South Africa, ART coverage in HIV-infected children increased from 2004, but by 2011, pediatric ART initiation rates still lagged behind that of adults. Nationally the 2011/12 coverage for children under 18 months was reported as 54.4% with large variations between districts [14, 19]. In our study, HIV-infected children still had an elevated risk of IPD-associated hospitalization (15-fold) and IPD related death (2-fold), compared to unexposed children. Other studies have shown that following ART introduction, although

overall incidence of IPD decreased in HIV-infected children [20], the absolute risk of IPD remained approximately 20-fold greater in HIV-infected than HIV-uninfected children under 2 years of age [21]. A case-control study from South Africa found that HIV exposure was associated with nearly two times greater odds of all serotype IPD, although the control group in this study was children hospitalized with a non-pneumonia diagnosis, a group also at increased likelihood of HIV exposure, and therefore this study likely underestimated the increased odds of IPD associated with HIV exposure [16].

In our study a number of differences were noted between HEU, HUU and HIV-infected IPD cases. Malnutrition was significantly more common in HIV-infected cases, but not in HEU when compared to HUU cases. This concurs with a review of studies that showed an association between HIV infection and being stunted or underweight [22]; no differences were observed in the early growth of HEU children and healthy controls [22]. Combined ART used for PMTCT has been shown to cause lower birth weights and lengths in some HEU infants, but this rapidly corrects over the first few months of life [23].

Other differences between the three IPD case groups included clinical presentation, with HUU IPD cases more likely to present with meningitis than pneumonia. HIV-infected children are less likely to be diagnosed with meningitis than other types of IPD [24] and children with meningitis have a higher mortality than children with pneumonia or bacteremia, especially if they are HIV infected [24]. Specimen taking practices differed between different case groups, reflecting different clinical syndromes; HUU were less likely to have blood cultures taken than HEU and HIV-infected cases.

Racial differences, with HUU children being more likely to be of non-black race has been shown in other local studies [25]. Children of black race had a higher likelihood of dying with IPD, possibly reflecting poorer socio-economic status and higher HIV-infection rates. IPD in HIV-infected individuals is more often caused by antibiotic resistant strains than IPD in HIV-uninfected individuals [26, 27]. Antimicrobial resistance is an important adverse consequence of cotrimoxazole prophylaxis [28]; this correlated with what we found in our study.

Cohort studies suggest that mortality among children born to HIV-infected mothers is higher than that among children born to HIV-uninfected mothers [29, 30]. A pooled mortality analysis, using African data, showed a 9 times higher mortality rate in HIV-infected than – uninfected children. Children with a early positive PCR result (<4 weeks of age) were more likely to die, as were those with mothers who died or who had low CD4+ cell counts at delivery [31]. In our study we observed a higher IPD-associated case fatality rate in HEU compared with HUU infants in the <6 month age group. The increased fatality rate among HEU children may be due to immunological differences that resolve as these children age; thus, younger HEU children may be more vulnerable to adverse clinical outcomes [32]. Other studies have also shown a higher mortality in younger HEU children [8]. In the older infants (6-<12 months) this relationship was reversed with HUU infants less likely to survive IPD, but there were small numbers of infants in the 6-<12 month HEU comparison group (n=78). By 6 months of age the immunologic deficit associated with HIV exposure is reduced [8] and effects of HIV exposure on adverse outcomes in this group are less marked. Lastly, increased case fatality rates in older HUU IPD cases may be due to a higher proportion of these cases having an underlying condition or possibly other factors leading to high

mortality in HUU IPD cases which we were not able to document. Children with underlying conditions have been extensively described to have a higher risk of IPD than healthy children [33]. HIV infection is an independent risk factor for IPD [34, 35]. This would account for the higher rate of underlying conditions in HEU and HUU IPD cases, compared with HIV-infected IPD cases. The difference in underlying conditions rates between HEU and HUU cases was not statistically significant and no solid conclusions could be made regarding this comparison.

A number of factors are thought to contribute to case fatality rate differences between HEU and HUU children. Most important are different immunological deficits documented in HEU children [36-39]. Secondly, a clear trend has been shown between the degree of maternal immunosuppression and infant survival [40]. We did not collect details regarding maternal CD4 count or use of antiretroviral treatment by the mother during pregnancy and could therefore not explore this association further which was a limitation.

Our study had other limitations. As with most surveillance studies only patients who had samples taken could be identified as an IPD case and included in the study. For the multinomial and mortality analyses we only included IPD cases from enhanced sites with viable isolates. These enhanced site cases were more likely diagnosed with positive blood cultures which may limit the generalizability of our findings. Infection status of HEU children was decided by one negative PCR result in some infants, so it is possible there may have been some misclassification of HIV status. The majority of patients had a PCR done within a month of admission and nurses were trained to request retesting in symptomatic children, which would have minimized HIV-infected children being included in the HEU group. Some

data, such as cotrimoxazole prophylaxis, was ascertained on verbal report if not available in the medical records, underreporting is therefore possible.

In conclusion we have described a higher incidence of hospitalized IPD in HEU children when compared to HUU children, as well as a lower chance of surviving IPD in HEU children <6 months of age compared with those who are HUU. Although we did not collect maternal data, we propose that optimizing maternal immunological status during pregnancy may help to improve outcomes in HEU children. While widespread PCV introduction has led to substantial reductions in IPD incidence in South Africa [15], some differences were observed in vaccination rates between HIV exposure groups. It is important to ensure that all HEU children receive PCV, to reduce the risk of IPD and its negative health outcomes, including death.

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Conflict of interest statement: CvM has received honoraria from Pfizer. AvG has received research funding from Pfizer. TA has received honoraria and conference support from Pfizer. GR has received honoraria and conference support from Pfizer and Sanofi. KLOB and KPK have received research funding and honoraria from Pfizer and GlaxoSmithKline. ST, SM, LdG, VQ, SL, NdP, BE, HF, MM, CGW and CC report no conflicts of interest.

"The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry."

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Supplementary appendix

1.1 List of collaborators

GERMS-SA (Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa)

Sandeep Vasaikar, Dania Perez (Eastern Cape); Eugene Elliot, Ute Hallbauer (Free State); Alan Karstaedt, Jeannette Wadula, Charl Verwey, Kathy Lindeque, Charlotte Sriruttan, Sharona Seetharam, Charles Feldman, Trusha Nana, Norma Bosman, Sheeba Varughese, Adrian Duse, Warren Lowman, David Moore, Charl Verwey, Mamokgethi Moshe, Kamaldeen Baba, Theunis Avenant, Nicolette du Plessis, Gary Reubenson, Ranmini Kularatne, Maphoshane Nchabeleng, Anwar Hoosen, Bonnie Maloba, Ruth Lekalakala (Gauteng); Yacoob Coovadia, Koleka Mlisana, Moherndran Archary, Ramola Naidoo, Khatija Dawood, Fathima Naby, Khine Sweswe, Prathna Bhola, Prasha Mahabeer, Lisha Sookan, Praksha Ramjathan, Halima Dawood, Sumayya Haffejee (Kwa-Zulu Natal); Ken Hamese, Phasweni Maredi, Takalani Muditambi (Limpopo) Greta Hoyland, Jacob Lebudi, Barry Spies (Mpumalanga); Stan Harvey, Pieter Jooste, Dhamiran Naidoo, Eunice Weenink (Northern Cape); Andrew Rampe, Lino Sono (North West); Elizabeth Wasserman, Preneshni Naicker, Andrew Whitelaw, Brian Eley, James Nuttal, Louise Cooke, Heather Finalyson, Helena Rabie, Collleen Bamford, Heidi Orth, Mark Nicol, Rena Hoffmann, Steve Oliver (Western Cape); Keshree Pillay, Chetna Govind, (LANCET); Adrian Brink, Maria Botha, Inge Zietsman, Inge Zietsman, Suzy Budavari, Xoliswa Poswa, Mark Cruz da Silva, Jennifer Coetzee (AMPATH); Marthinus Senekal (PATHCARE); Chris van Beneden, Stephanie Schrag, Elizabeth Zell, Anne Schuchat, Tom Chiller, Angela Ahlquist, Fred Angulo,(CDC); Keith Klugman, (Emory); Katherine O'Brien (Johns Hopkins Bloomberg School of Public Health); Anne von Gottberg, Linda de Gouveia, Mignon du Plessis, Karen Keddy, Arvinda Sooka, Nelesh Govender,

Jaymati Patel, Vanessa Quan, Susan Meiring, Melony Fortuin-de Smidt, Mohlamme John Mathabathe, Claire von Mollendorf, John Frean, Desiree du Plessis, Bhavani Poonsamy, Olga Perovic, Marshagne Smith, Cheryl Cohen, Penny Crowther, Jabulani Ncayiyana, Relebohile Ncha, Languta Sibiya, Sonwabo Lindani, Nevashan Govender, Nireshni Naidoo, Babatyi Kgokong, Vusi Nokeri, Sarona Lengana (NICD); Ntombenhle Ngcobo, Johann van den Heever (National Department of Health, Expanded Programme on Immunisation, Pretoria), Shabir Madhi (Department of Science and Technology/ National Research Foundation: Vaccine Preventable Diseases, Gauteng), Laura Conklin, Jennifer Verani, Cynthia Whitney, Elizabeth Zell, Jennifer Loo, George Nelson (National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta)

1.2 Methods

Study design and setting

For each non-enhanced case a laboratory report form (with information on age, gender, date of specimen collection and source of specimen) and the associated pneumococcal isolate is submitted to the National Institute for Communicable Diseases (NICD), Johannesburg, South Africa. Additional clinical and demographic information collected at enhanced sites includes admission date, HIV exposure and infection status, discharge diagnosis, vaccination status and outcome through patient interview and medical record review.

Case definitions

Laboratory testing for pneumococcus was performed as part of routine medical care. Only IPD cases diagnosed by positive culture or polymerase chain reaction (PCR), or by latex agglutination test with supporting evidence (Gram stain or PCR positive) were included.

HIV infection and exposure status was defined according to the most recent HIV PCR (for HIV-infected, HEU or HUU children) or ELISA (for HUU children). In addition to the review of admission records, a search was also conducted using the laboratory data system for updated HIV and if applicable CD4 results for children included in the study. If the child was not tested on admission, the 6 week PCR result for exposed children was used unless they had any signs/symptoms suggestive of HIV. If breastfeeding information was available it also informed the interpretation of results. If the child was symptomatic for HIV, surveillance officers worked with the ward doctors to retest the children at the current admission. Final HIV exposure and HIV infection status was decided by individual case review by a medical officer (CvM) taking into account all available HIV results and breastfeeding history. Cases in which a clear determination of HIV exposure status could not be made based on available information were classified as HIV status unknown.

Malnutrition was defined according to the World Health Organization (WHO) child growth standards. Malnourished infants included those with weight-for-age Z-scores less than minus two standard deviations or nutritional edema. Underlying conditions included asplenia; chronic illness, including chronic lung, renal, liver and cardiac disease; other immunocompromising conditions (excluding HIV); and other risk factors, including head injury with possible CSF leak, neurological disorders, burns and chromosomal abnormalities,

but excluded malnutrition. Clinical diagnoses were based on documented discharge diagnoses in the medical records with clinical syndrome, being defined as meningitis, bacteremic pneumonia, and bacteremia without focus/other. Pitt bacteremia score was calculated using (1) oral temperature, (2) hypotension, (3) receipt of mechanical ventilation, (4) cardiac arrest and (5) mental status. Severe disease was defined as a score of ≥4 points [1]. In-hospital outcome was defined as recovered (discharged) or died during in-hospital stay. A case was considered to be recurrent if pneumococcal disease was diagnosed in the same patient more than 21 days after the first confirmed laboratory diagnosis of *S. pneumoniae* disease. Cotrimoxazole prophylaxis is administered in HEU and HIV-infected children for differing time periods to prevent PCP and is not given to HUU children. We therefore included this variable *a priori* in our analysis as it could confound other associations.

Penicillin non-susceptibility was categorized using the 2010 Clinical and Laboratory Standards Institute breakpoints for oral penicillin V (susceptible, ≤0.06mg/L; intermediately resistant, 0.12-1mg/L and resistant, ≥2mg/L) [2]. The intermediately resistant and resistant groups were combined into a non-susceptible group for analysis. Vaccine-serotype IPD was defined as serotypes present in the 13-valent pneumococcal conjugate vaccine (PCV-13) (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). All other serotypes were designated non-vaccine types.

Incidence

Denominators for HIV-infected infants <1 year of age were calculated by combining denominators for new HIV infection at/before birth and new HIV infection due to

breastfeeding in infants <1 year. Denominators for HEU infants were calculated from the population denominator for HIV-infected pregnant women, adjusted for live births and less HIV-infected infants <1 year of age and infants who were infected postnatally.

Factors associated with HIV exposure status and death

We assessed all variables significant at P<0.2 on univariate analysis and eliminated non-significant factors (p≥0.05) with stepwise backward selection from the models. Patients with missing data for included variables were excluded. In addition, for the mortality analyses we excluded variables considered to be on the causal pathway for mortality (i.e. length of hospital stay, Pitt bacteremia score and antibiotic treatment received in the 24 hours prior to admission) since anything on the causal pathway is not an independent risk factor.

1.3 Tables

Supplementary Table 1: Univariate and multivariate multinomial logistic regression model showing comparison of demographic, socio-economic characteristics, and underlying conditions in HIV-exposed-uninfected (HEU), HIV-unexposed-uninfected (HUU) and HIV-infected (HI) IPD cases <1 year of age, at enhanced GERMS-SA sites in South Africa, 2009-2013 (n=937)

	HEU cases Reference	HUU cases			HI cases		
	n/N (%)	n/N (%)	RRR[a] (95%CI)	ARRR[b] (95%CI)	n/N (%)	RRR[a] (95%CI)	ARRR[b] (95%CI)
Demographics and socioeconomic characteristics							
Age ≥ 6 months	84/273 (31.0)	158/407 (38.8)	1.43 (1.03-1.98)	1.82 (1.17-2.84)	123/257 (47.9)	2.07 (1.45-2.95)	2.71 (1.67-4.38)
Male Sex	147/273 (53.9)	229/407 (56.3)	1.10 (0.81-1.50)		138/256 (53.9)	1.00 (0.71-1.41)	
Black Race	250/257 (97.3)	335/393 (85.2)	0.16 (0.07-0.36)	0.13 (0.05-0.36)	243/247 (98.4)	1.70 (0.49-5.88)	1.39 (0.32-5.99)
Length of hospital stay:							
<4 days	65/251 (25.9)	85/376 (22.6)	Reference		65/244 (26.6)	Reference	
4-14 days	129/251 (51.4)	170/376 (45.2)	1.01 (0.68-1.50)		113/244 (46.3)	0.88 (0.57-1.34)	
≥15 days	57/251 (22.7)	121/376 (32.2)	1.62 (1.03-2.55)		66/244 (27.1)	1.16 (0.71-1.90)	
Medical conditions, treatment and vaccination status							
Underlying conditions[c]	36/228 (15.8)	63/350 (18.0)	1.17 (0.75-1.83)	1.31 (0.73-2.35)	15/216 (6.9)	0.40 (0.21-0.75)	0.30 (0.14-0.63)
Malnutrition[d]	89/246 (36.2)	135/368 (36.7)	1.02 (0.73-1.43)		153/231 (66.2)	3.46 (2.37-5.04)	

Previous hospital admission in last 12 months	51/246 (20.7)	99/372 (26.6)	1.39 (0.94-2.04)		92/230 (40.0)	2.55 (1.70-3.82)	
In-hospital mortality	74/253 (29.3)	94/377 (24.9)	0.80 (0.56-1.15)	1.06 (0.65-1.74)	81/242 (33.5)	1.22 (0.83-1.78)	2.03 (1.18-3.49)
Pitt bacteremia score (≥4)[e]	35/245 (14.3)	49/367 (13.4)	0.92 (0.58-1.48)		20/237 (8.4)	0.55 (0.31-0.99)	
Antibiotics in last 24 hours[f]	20/240 (8.3)	49/362 (13.5)	1.72 (1.00-2.98)		24/223 (10.8)	1.33 (0.71-2.48)	
Antibiotics in last 2 months [g]	24/235 (10.2)	69/365 (18.9)	2.05 (1.25-3.37)		45/212 (21.2)	2.39 (1.39-4.05)	
Previous IPD infection [h]	4/273 (1.5)	12/407 (3.0)	2.04 (0.65-6.40)		15/257 (5.8)	4.16 (1.36-12.73)	
Cotrimoxazole prophylaxis	34/243 (14.0)	0/407 (0.0)	Not calculated	Not calculated	87/221 (39.4)	3.99 (2.54-6.27)	4.56 (2.63-7.89)
Treated for tuberculosis	9/249 (3.6)	10/377 (2.7)	0.73 (0.29-1.81)		30/228 (13.2)	4.04 (1.87-8.71)	
Vaccination status [i]							
- 0 doses	115/238 (48.3)	134/339 (39.5)	Reference		62/173 (35.8)	Reference	
- 1 dose	64/238 (26.9)	98/339 (28.9)	1.31 (0.88-1.96)		47/173 (27.1)	1.36 (0.84-2.22)	
- 2 doses	59/238 (24.8)	107/339 (31.6)	1.56 (1.03-2.33)		64/173 (37.0)	2.01 (1.26-3.22)	
Pneumococcal isolate characteristics							
Penicillin non-susceptible [j]	99/219 (45.2)	137/331 (41.4)	0.86 (0.61-1.21)	0.74 (0.49-1.11)	138/216 (63.9)	2.14 (1.46-3.15)	1.66 (1.04-2.65)
Vaccine serotypes[k]	139/244 (57.0)	198/356 (55.6)	0.95 (0.68-1.31)		160/225 (71.1)	1.86 (1.27-2.73)	
Clinical syndrome and specimen type							
Clinical syndrome							

- Pneumonia	115/266 (43.2)	141/395 (35.7)	Reference	Reference	124/249 (49.8)	Reference	Reference
- Meningitis	118/266 (44.4)	188/395 (47.6)	1.30 (0.93-1.82)	1.61 (1.04-2.50)	94/249 (37.8)	0.74 (0.51-1.07)	0.72 (0.44-1.19)
- Bacteremia	33/266 (12.4)	66/395 (16.7)	1.63 (1.01-2.65)	1.67 (0.88-3.18)	31/249 (12.5)	0.87 (0.50-1.51)	1.05 (0.51-2.15)
Specimen type[l]							
- Blood culture	166/273 (60.8)	207/407 (50.9)	Reference		167/257 (65.0)	Reference	
- Cerebrospinal fluid	103/273 (37.7)	175/407 (43.0)	1.36 (0.99-1.87)		84/257 (32.7)	0.81 (0.57-1.16)	
- Other	4/273 (1.5)	25/407 (6.1)	5.01 (1.71-14.69)		6/257 (2.3)	1.49 (0.41-5.38)	

[a]Relative risk ratio; [b]Adjusted relative risk ratio; [c]Asplenia, including asplenia or sickle cell anemia; chronic illness, including chronic lung, renal, liver, cardiac disease and diabetes; other immunocompromising conditions (excluding HIV), including organ transplant, primary immunodeficiency, immunotherapy and malignancy; and other risk factors, including head injury with possible CSF leak, neurological disorders, burns and chromosomal abnormalities. Excludes malnutrition; [d]Malnutrition was classified as children with weight-for-age Z-score < -2 (WHO child growth standards 2009) and/or children with nutritional edema; [e]Pitt bacteremia score calculated using temperature, hypotension, mechanical ventilation, cardiac arrest and mental status. Severe disease defined as score of ≥4 points; [f]Any antibiotics used in 24 hours prior to admission; [g]Any antibiotics used in 2 months prior to admission; [h]Previously diagnosed with IPD (invasive pneumococcal disease) more than 21 days prior to this episode; [i]Vaccination status determined only for cases eligible to have received the pneumococcal conjugate vaccine; [j]Penicillin non-susceptible MIC ≥ 0.12 µg/mL; [k]Vaccine serotypes were considered as serotypes in the 13-valent pneumococcal conjugate vaccine; [l]Elected to use clinical diagnosis rather than specimen type in multivariable model

1.4 References

1. Paterson DL, Ko WC, von Gottberg A, et al. Antibiotic therapy for *Klebsiella pneumoniae* bacteremia: implications of production of extended-spectrum beta-lactamases. ClinInfect Dis **2004**; 39(1): 31-7.
2. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twentieth informational supplement. Wayne, PA, **2010** 2010. Report No.: CLSI document M100-S20.